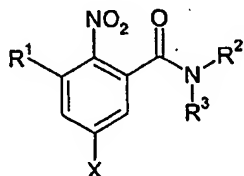




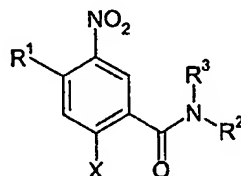
INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁷ : C07C 233/66, 233/65, 235/38, C07K 14/705, A61P 5/00, C07D 221/14	A1	(11) International Publication Number: WO 00/55118 (43) International Publication Date: 21 September 2000 (21.09.00)
(21) International Application Number: PCT/US00/06537 (22) International Filing Date: 13 March 2000 (13.03.00) (30) Priority Data: 60/124,635 16 March 1999 (16.03.99) US (71) Applicant (for all designated States except US): GLAXO GROUP LIMITED [GB/GB]; Glaxo Wellcome House, Berkeley Avenue, Greenford, Middlesex UB6 0NN (GB). (72) Inventors; and (75) Inventors/Applicants (for US only): BLANCHARD, Steven, Gerard [US/US]; Glaxo Wellcome Inc., Five Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709 (US). COBB, Jeffery, Edmund [US/US]; Glaxo Wellcome Inc., Five Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709 (US). COLLINS, Jon, Loren [US/US]; Glaxo Wellcome Inc., Five Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709 (US). WILLSON, Timothy, Mark [GB/US]; Glaxo Wellcome Inc., Five Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709 (US). (74) Agents: LEVY, David, J.; Glaxo Wellcome Inc., Five Moore Drive, P.O. Box 13398, Research Triangle Park, NC 27709-3398 (US) et al.	(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	

(54) Title: NUCLEAR RECEPTOR ARYLATING COMPOUNDS



(IA)



(IB)

(57) Abstract

The present invention discloses novel nuclear receptor ligands of formula (IA) or (IB). These compounds are useful for arylating a cysteine in a nuclear receptor.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon	KR	Republic of Korea	PL	Poland		
CN	China	KZ	Kazakhstan	PT	Portugal		
CU	Cuba	LC	Saint Lucia	RO	Romania		
CZ	Czech Republic	LI	Liechtenstein	RU	Russian Federation		
DE	Germany	LK	Sri Lanka	SD	Sudan		
DK	Denmark	LR	Liberia	SE	Sweden		
EE	Estonia			SG	Singapore		

Nuclear Receptor Arylating Compounds

The present invention relates to compounds that bind to and arylate nuclear receptors. In another aspect, the present invention relates to methods for associating a particular disease or condition with a particular nuclear receptor.

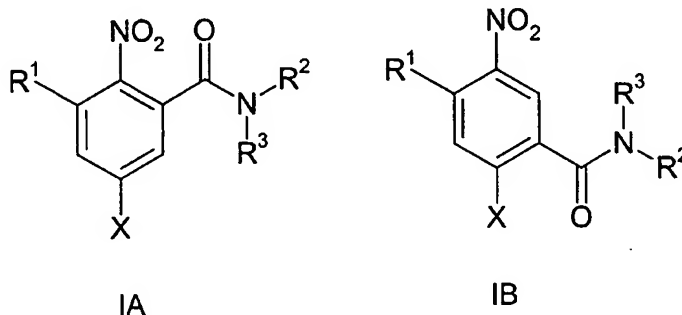
Nuclear receptors are transcription factors belonging to the steroid/retinoid receptor superfamily that are activated or deactivated by small lipophilic hormones. See, for example Mangelsdorf, D. J. et al., *Cell*, (1995), 83, 835-839. The Peroxisome Proliferator Activated Receptors (PPARs) are orphan members of the nuclear receptor superfamily. See, for example, Willson, T. M. and Wahli, W., *Curr. Opin. Chem. Biol.*, (1997), Vol. 1, pp 235-241.

Three mammalian PPARs have been identified which are termed PPAR-alpha, PPAR-gamma, and PPAR-delta. PPARs regulate expression of target genes by binding to DNA response elements as heterodimers with the retinoid X receptor. These DNA response elements (PPRE) have been identified in the regulatory regions of a number of genes encoding proteins involved in lipid metabolism and energy balance. The biological role of the PPARs in the regulation of lipid metabolism and storage has been recently reviewed. See, for example, Spiegelman, B. M., *Diabetes*, (1998), Vol. 47, pp 507-514, Schoonjans, K., Martin, G., Staels, B., and Auwerx, J., *Curr. Opin. Lipidol.*, (1997), Vol. 8, pp 159-166, and Brun, R. P., Kim, J. B., Hu, E., and Spiegelman, B. M., *Curr. Opin. Lipidol.*, (1997), Vol. 8, pp 212-218.

Essential dietary fatty acids and certain of their eicosanoid metabolites are naturally-occurring hormones for the PPAR receptors. These hormones can promote adipogenesis through activation of the PPAR-gamma receptor. See, for example, Kliewer, S. A., et al., *Proc. Natl. Acad. Sci. USA*, (1997), Vol. 94, pp 4318-4323, and Kliewer, S. A., et al., *Cell*, (1995), Vol. 83, pp 813-819. Molecules that inhibit the adipogenic effects of endogenous PPAR-gamma hormones may be useful in the treatment of diseases caused by

increased fat accumulation or lipid storage. See, for example, Tontonoz, P., Hu, E., and Spiegelman, B. M., Curr. Opin. Genet. Dev., (1995), Vol. 5, pp 571-576. Examples of these diseases are obesity, osteoporosis, and acne.

5 Briefly, in one aspect, the present invention discloses compounds of formula IA or IB,



where X is halogen;

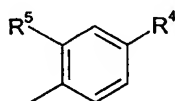
R¹ is H or -OCH₃;

10 R² is a hydrophobic organic group with molecular weight less than 500 Daltons; and

R³ is H, C₁₋₆alkyl, or phenyl optionally substituted with 1 or 2 groups selected from C₁₋₃alkyl, C₁₋₃alkoxy, and halogen. Preferably, the compounds of this invention are capable of binding to the ligand-binding domain of a nuclear
15 receptor and arylating a cysteine residue of the receptor.

Preferably, X is Cl, F, or Br. Most preferably, X is Cl.

Preferably, R² is



or a -C₁₋₃alkenyl-adamantyl, where R⁴ and R⁵ are independently hydrogen or halogen. Most preferably, R⁴ and R⁵ are both H, both chloro, or one is H and
20 one is iodo.

Preferably, R³ is hydrogen, mono-substituted or un-substituted phenyl, or C₁₋₃alkyl. Most preferably, R³ is H.

In another aspect, the present invention discloses a method of inhibiting the activity of a nuclear receptor by arylating a cysteine residue within the ligand-binding domain of said receptor.

5 In another aspect, the present invention discloses a method for associating a particular disease or condition with a particular nuclear receptor, for example PPARgamma. By "associating" is meant that the particular disease or condition can be treated by the administration of a compound that activates or deactivates the particular nuclear receptor.

10 In another aspect, the present invention provides a method for the treatment of a nuclear receptor mediated disease or condition by administration of a compound that activates or deactivates the particular nuclear receptor that was associated with said disease or condition using the method of this invention.

15 Preferred compounds of this invention are capable of binding to the ligand-binding domain of a nuclear receptor and arylating a cysteine residue of the receptor. Using well-known techniques compounds of formula IA or IB can be prepared, tested for binding to a particular receptor and analysed for arylation of the receptor. For example, a library of compounds of formula IA or IB can be prepared. The library can then be screened using a binding
20 assay to identify members of the library that show affinity to a particular nuclear receptor, for example PPAR-gamma. Compounds that bind with a suitable apparent pKi, for example with an apparent pKi > 5, preferably > 7, would then be good candidates for arylating compounds. Arylation of
25 cysteine residues within the nuclear receptor ligand-binding domain can be demonstrated by standard biochemical techniques, for example mass spectral analysis. Compounds that are shown to bind and to arylate, can be screened for selectivity using binding assays for other nuclear receptors, for example RXR. Generally this arylation of a cysteine in the ligand binding
30 domain will block the functional activity of a nuclear receptor. This expected blocking could be confirmed using a cell-based reporter assay.

Arylating compounds that bind to a nuclear receptor and therefore block its functional activity in a cell line, cell culture, tissue, or whole animal can be used to associate the nuclear receptor with a mammalian disease.

5 For example, arylating compounds that block PPAR-gamma activity inhibit adipogenesis, thereby suggesting that PPARgamma plays a role in adipogenesis. Although some nuclear receptors are associated with particular diseases or conditions, there may be more diseases or conditions that can be treated or prevented by a compound that activates or deactivates that receptor. Some nuclear receptors have no known utility. By using the associating methods of this invention, new disease-receptor associations can be discovered. Once these new associations are discovered, then new drugs for these diseases can be discovered by searching for compounds that activate or deactivate the receptor. Thus, the present invention provides new methods for drug discovery, and consequently new drugs for prevention and treatment of human diseases and conditions.

Suitable compounds of the present invention include:

N-phenyl-2-chloro-5-nitro-benzamide,
N-(2,4-dichlorophenyl)-2-chloro-5-nitro-benzamide,
20 N-(4-iodophenyl)-2-chloro-5-nitro-benzamide,
N-phenyl-5-chloro-3-methoxy-2-nitro-benzamide,
N-phenyl-2-fluoro-5-nitro-benzamide,
N-(1-tricyclo[3.3.1.1^{3,7}]dec-1-ylethyl)-2-chloro-5-nitro-benzamide, and
N-(2-ethoxyphenyl)-N-(6-bromo-1H-benz[de]isoquinoline-1,3(2H)-dioxo-eth-2-yl)-2-chloro-5-nitro-benzamide.

25 A particularly preferred compound of the present invention is N-phenyl-2-chloro-5-nitro-benzamide.

It will be appreciated by those skilled in the art that reference herein to treatment extends to prophylaxis as well as the treatment of established diseases or symptoms.

30

As used herein, "alkyl", "alkenyl", and similar terms and terms containing these terms, include straight-chain and branched-chain alkyl chains unless otherwise indicated.

5 The compounds of this invention can be prepared by standard organic chemistry as illustrated by the accompanying working examples. The following examples are set forth to illustrate the synthesis of some particular compounds of the present invention and to exemplify general processes. Accordingly, the following Examples section is in no way intended to limit the
10 scope of the invention contemplated herein.

Examples

Example 1: N-phenyl-2-chloro-5-nitro-benzamide

15 To a stirred solution of 2-chloro-5-nitro-benzoyl chloride (5.03 g, 22.9 mmol) and triethylamine (3.51 mL, 25.1 mmol) in CH₂Cl₂ maintained under nitrogen at 0°C was added dropwise aniline (2.19 mL, 24.0 mmol). The resulting solution was stirred for 5 minutes at 0°C and then for 15 minutes at room temperature. This solution was then diluted with EtOAc (300 mL) and
20 washed sequentially with 1.0 M HCl, water, 1.0 M NaHCO₃ and brine (100 mL each). The organic solution was then dried over MgSO₄ and concentrated by rotary evaporation to give a light yellow solid (5.32 g) which was recrystallized from EtOAc to provide the title compound as a white solid (3.34 g, 53%): mp 155-156 °C; ¹H NMR (CDCl₃, 400 MHz) δ 8.63 (d, 1H, J=2.7), 8.28 (dd, 1H, J=2.7, 8.9), 7.81 (br s, 1H) 7.68-7.63 (m, 3H), 7.42 (t, 2H, J=7.9), 7.23 (t, 1H, J=7.5); MS (ES⁻) *m/e* 275.1 (M-H)⁻, Anal. Calcd. for C₁₃H₉ClN₂O₃: C, 56.43; H, 3.28; N, 10.13; Found: C, 56.33; H, 3.30; N, 10.03.

Examples 2-5

30 In a similar manner to the preparation of Example 1, the following examples were prepared.

Example 2 N-(2,4-dichlorophenyl)-2-chloro-5-nitro-benzamide

- Example 3 N-(4-iodophenyl)-2-chloro-5-nitro-benzamide
Example 4 N-phenyl-5-chloro-3-methoxy-2-nitro-benzamide
Example 5 N-phenyl-2-fluoro-5-nitro-benzamide

5

Binding assay

Test compounds were assayed for binding to the human PPAR-
gamma receptor ligand binding domain by scintillation proximity assay (SPA)
as described in Nichols, J. S., Parks, D. J., Consler, T. G., and Blanchard, S.
10 G., Anal. Biochem., Vol. 257, pp.112-119, and Vol. 263, p 126 (1998),. Each
of the above Examples 1-5 had an apparent $pK_i > 7$ in this binding assay.

Binding to the PPAR- α and PPAR- δ ligand binding domains was
determined in a similar manner using the previously described radioligands
15 [3H]-GW2331 (Kliwer, S.A. *et. al.*, and Lehmann, J.M. Proc. Natl. Acad Sci
USA (1997) Vol.94 pp. 4318-4323) and [3H]-GW2433 (Brown, P.J., Smith-
Oliver, T. A., Charifson, P.S., Tomkinson, N.C.O., Fivush, A.M., Sternbach,
D.D., Wade, L.E., Orband-Miller, L., Parks, D.J., Blanchard, S.G., Kliwer,
S.A., Lehmann, J.A. and Willson, T.M. Chemistry and Biology (1997) Vol. 4,
pp.909-918) for PPAR- α and PPAR- δ , respectively.

20 The buffer used for the PPAR- γ and PPAR- α SPA was 50 mM KCl, 2
mM EDTA, 5 mM CHAPS, 0.1 mg/mL BSA, 10 mM DTT, and 50 mM Tris (2-
amino-2-hydroxymethyl-1,3-propanediol) pH 8 (Buffer A). For the PPAR- δ
SPA, the Tris was replaced with HEPES (N-[2-Hydroxyethyl]piperazine-N'-[2-
ethanesulfonic acid]) and the pH was 7 (Buffer B).

25 Analysis of the apparent binding affinity of the compound of Example
1, using standard scintillation proximity assays for PPAR γ , PPAR α , and
PPAR δ , showed that the compound is a selective ligand for PPAR γ .

Compared to its binding to PPAR γ , the compound bound to PPAR α and
PPAR δ with ~10-fold and ~600-fold lower affinity. Apparent pK_i s of $8.48 \pm$
30 0.27 ($K_i=3.3$ nM; $n=10$), 7.49 ± 0.17 ($K_i=32$ nM; $n=9$), 5.69 ± 0.17 ($K_i=2000$
nM; $n=3$), were observed for binding to for PPAR γ , PPAR α , and PPAR δ ,
respectively.

Cell-based report r assay

CV-1 cells were maintained in DME High Glucose medium (Irvine Scientific) supplemented with 10% fetal bovine serum and 2 mM Glutamine.

5 Cells were split into D-MEM/F-12 medium (Gibco) supplemented with 10 % charcoal stripped fetal bovine serum for 3 d before harvesting. Cells were harvested into D-MEM/F-12 medium (Gibco) supplemented with 10 % charcoal stripped fetal bovine serum and counted. Cells were seeded at a density of 24,000 cells per well into 96-well plates and incubated overnight at

10 5% CO₂ and 37 °C. Cells were transfected for 6 to 20 hours based on the Lipofectamine protocol (Gibco) with the following amounts of DNA per well: 2 ng PSG5 GAL4-human PPAR-gamma, 8 ng UAS-tk-SPAP, 25 ng beta-gal, 45 ng pBluescript. See Lehmann, J. M. et al., J. Biol. Chem., (1995), Vol. 270, pp 12953-12956 and Brown, P. J. et al., Chem. Biol., (1997), Vol. 4, pp

15 909-918. Cells were incubated overnight at 5 % CO₂ and 37 °C. Test compounds were solublized to 10 mM in DMSO. Test compounds were then serially diluted from 1e-5 M to 1e-10 M into D-MEM/ F-12 (Gibco) medium supplemented with 10% delipidated and charcoal stripped calf serum (Sigma) heat inactivated at 60 °C for 30 minutes, 2 mM Glutamine, and Pen-Strep.

20 This medium into which the test compounds were diluted also contained 100 nM rosiglitazone. These test compound dilutions were added 100 microliters/well to the transfected cell plates after the transfection media were aspirated. DMSO controls and 1 micromolar rosiglitazone controls were added to each cell plate. Cells were incubated overnight at 5 % CO₂ and 37

25 °C. Cells were lysed with 25 microliters 0.5 % Triton X-100. Two daughter plates were made from each mother plate. One daughter received 200 microliters/well SPAP substrate (Sigma 104) and the other daughter received 200 microliters/well beta-gal substrate (Sigma N-1127). Once developed, cell plates were read at 405 nM. SPAP data were normalized to beta-gal, and %

30 maximum inhibition of transactivation was calculated relative to the 1 micromolar rosiglitazone positive control. Each of the above Examples 1-5

had >50 % inhibition of transactivation by 100 nM rosiglitazone in this PPAR-gamma cell based reporter gene assay.

Blocking PPAR gamma Binding

5 PPAR γ (in Buffer A) immobilised on SPA beads was incubated with 1 μ M of the compound of Example 1 (■ in the graph below) or rosiglitazone (● in the graph below) for 1 hour. (Buffer A was used throughout this experiment.) This pretreating ligand was removed by two sequential steps of centrifugation followed by resuspension of the beads in fresh buffer.

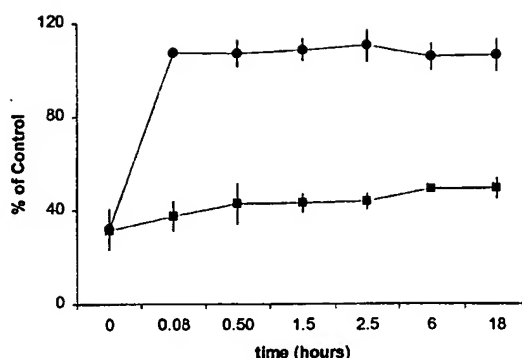
10 Recovery of binding was assessed by addition of [3 H]-rosiglitazone to an aliquot of the washed bead suspension followed by scintillation proximity assay to monitor the recovery of radioligand binding as a function of time. Biotinylated receptor immobilized on streptavidin-coated SPA beads was exposed either to vehicle, 1 μ M the compound of Example 1, or to

15 rosiglitazone. Following incubation for one hour, the pretreating ligand was removed from immobilized receptor and radioligand was added. Recovery of binding as a function of time was assessed by SPA. When PPAR γ was treated with the compound of Example 1 in this manner, persistent inhibition of [3 H]-rosiglitazone binding to the receptor was observed. Little recovery of

20 binding activity was observed at times up to 24 hours after removal of pretreating compound, suggesting that the inhibition was irreversible. In contrast, full receptor binding activity was recovered in parallel experiments where unlabeled rosiglitazone replaced the compound of Example 1 in the incubation. The recovery was rapid, reaching a maximum five minutes after

25 dilution of the pretreating ligand. These results are summarised in the graph below.

9



5

The standard conditions for the PPAR SPAs included 10 mM of the reducing agent dithiothreitol (DTT) in the assay buffers. The purpose of the DTT was two-fold. First, experiments had shown that maximal ligand binding was observed in the presence of reducing agent and that treatment of receptor with cysteine modifying reagents such as iodoacetamide inhibited radioligand binding. Therefore DTT reducing agent was added to increase ligand binding.

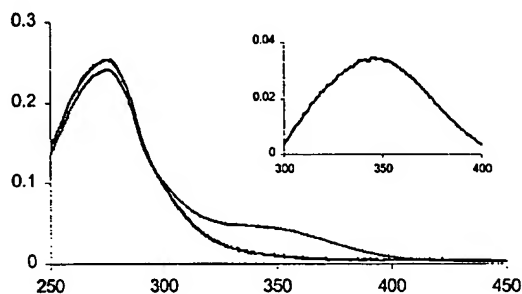
The second reason for use of DTT in the assay buffer was to act as a scavenger for nonspecific protein modifying reagents. As the concentration of DTT was more than six orders of magnitude above the concentration of receptor protein in the assay (~5 nM) the possibility of *nonspecific* receptor modification seemed unlikely.

The X-ray crystal structure of the PPAR γ ligand binding domain (LBD) shows that the single cysteine residue in the LBD is located in helix 3, which defines part of the ligand binding site. See, R. T. Nolte et al, *Nature*, Vol. 395, pp 137-143 (1998). The following UV-Visible absorption study and LC-Mass Spec. Studies are designed to study the effect of compounds on this cysteine.

25

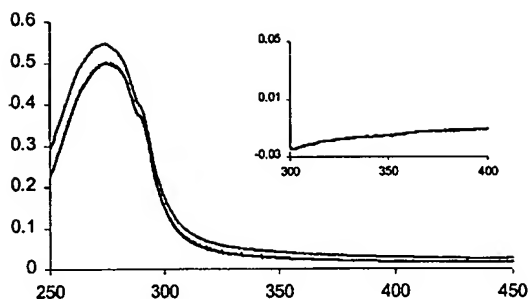
UV-Visibl Absorption

Covalent modification of reactive residues in proteins with nitro aryl halides such as dinitrofluorobenzene results in an increase in absorption at 330-360 nm. See, Means and Feeney, Chemical Modification of Proteins, Chapter 7, pp 118-123 (Holden-Day 1971). In the following experiments, spectra (in Buffer A) of the receptor protein and the compound of Example 1 were obtained separately. Backgrounds were subtracted and the two spectra were added together to give a composite spectrum for the unreacted sample (lower curve in each panel). The compound of Example 1 was then added to the receptor sample and a spectrum of the treated receptor was obtained (upper curves). As shown below, treatment of 5 μ M PPAR γ LBD with 5 μ M (one equivalent) of the compound of Example 1 resulted in an increase in absorbance centered at 350 nm, consistent with covalent modification of the protein. The absorbance change was rapid; the maximal increase occurred in the time required to mix the sample and begin recording of the spectrum.



PPAR Gamma and Compound of Example 1

In contrast to the results observed with PPAR γ , as shown below, no change in absorbance was detected on treatment of 10 μ M of the LBD of the unrelated Estrogen Receptor β with 10 μ M of the compound of Example 1.



Estrogen Receptor and Compound of Example 1

5

LC-Mass Spec.

In order to directly demonstrate that treatment with the compound of Example 1 resulted in covalent modification of PPAR γ , a sample of receptor was exposed to the compound and subjected to high-pressure liquid chromatography – electrospray ionization mass spectrometry. A second, untreated sample of receptor served as control. The treated sample showed an increase in molecular weight relative to the control that corresponded to the molecular weight of the compound of Example 1 minus HCl. Samples of the modified PPAR γ were subjected to digestion by the proteolytic enzyme Glu-C. Sequencing of the resulting peptides using mass spectrometry techniques confirmed Cys as the site of modification.

10
15

Adipocyte differentiation (adipogenesis) assay

C3H10T1/2 clone 8 murine fibroblasts (American Type Culture Collection) below passage 22 were maintained in Dulbecco's modified Eagle's medium (Life Technologies, Inc.) supplemented with 10 % fetal calf serum and 100 units/mL penicillin G and 100 microgram/mL streptomycin. One day after passage into 96-well microtiter plates (12.5×10^3 cells/cm 2), the cells were treated with 150 nM rosiglitazone plus 1 micromolar insulin and 1 micromolar 9-cis-retinoic acid (Sigma, St. Louis, Mo). Vehicle or test compounds, which had been solubilized to 10 mM in DMSO and then serially diluted from 1e-5 M to 1e-10 M into medium, were added. After 7 days, cells

20

25

were lysed in 0.01% Digitonin (Sigma, St. Louis, Mo) and the lipogenic activity determined by measuring total triglycerides using a Glycerol-Triglyceride (GPO-Trinder) kit (337-B, Sigma, St. Louis, Mo). The mixture was incubated
5 at 37 °C for 2 h and the absorbance read at 550 nm. The % maximum inhibition of lipogenesis was calculated relative to the vehicle treated cells. Example 1 had > 50 % inhibition of lipogenesis induced by 150 nM rosiglitazone in this adipocyte differentiation assay.

10 The following two examples were prepared and are useful for binding to and arylating CAR and LXR, respectively.

Example 6: N-(1-tricyclo[3.3.1.1^{3,7}]dec-1-ylethyl)-2-chloro-5-nitro-benzamide

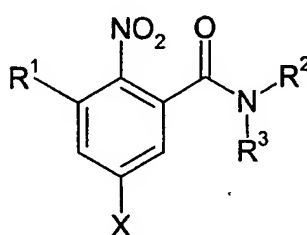
The title compound was prepared and is useful for binding to and
15 arylating CAR. For information on CAR, see, for example, Forman, B. M. et al., Nature (1998), 395, 612-615.

Example 7: N-(2-ethoxyphenyl)-N-(6-bromo-1H-benz[de]isoquinoline-1,3(2H)-dioxo-eth-2-yl)-2-chloro-5-nitro-benzamide

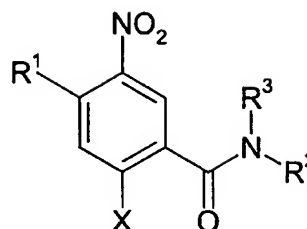
20 The title compound was prepared and is useful for binding to and arylating LXR. For information on LXR, see, for example, Peet, D. J. et al., Curr. Opin. Genet. Dev. (1998), 8, 571-575.

What is claimed is:

1. A compound of formula IA or IB,



IA



IB

5

where X is halogen;

R¹ is H or -OCH₃;

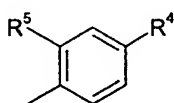
R² is a hydrophobic organic group with molecular weight less than 500 Daltons; and

- 10 R³ is H, C₁₋₆alkyl, or phenyl optionally substituted with 1 or 2 groups selected from C₁₋₃alkyl, C₁₋₃alkoxy, and halogen.

2. The compound of Claim 1 wherein X is Cl, F, or Br.

15

3. The compound of Claim 1 wherein R² is



or a -C₁₋₃alkenyl-adamantyl, where R⁵ and R⁴ are independently hydrogen or halogen.

20

4. The compound of Claim 3 wherein R⁵ and R⁴ are both are H, both chloro, or one is H and one is iodo.

5. The compound of Claim 1 wherein R³ is hydrogen, mono-substituted or un-substituted phenyl, or C₁₋₃alkyl.

5 6. The compound of Claim 1 wherein R³ is H.

7. The compound of Claim 1 wherein said compound is selected from the group consisting of

N-phenyl-2-chloro-5-nitro-benzamide,
10 N-(2,4-dichlorophenyl)-2-chloro-5-nitro-benzamide,
N-(4-iodophenyl)-2-chloro-5-nitro-benzamide,
N-phenyl-2-chloro-4-methoxy-5-nitro-benzamide,
N-phenyl-2-fluoro-5-nitro-benzamide,
N-(1-tricyclo[3.3.1.1^{3,7}]dec-1-ylethyl)-2-chloro-5-nitro-benzamide, and
15 N-(2-ethoxyphenyl)-N-(6-bromo-1H-benz[de]isoquinoline-1,3(2H)-dioxo-eth-2-yl)-2-chloro-5-nitro-benzamide.

8. The compound of Claim 1 wherein said compound is N-phenyl-2-chloro-5-nitro-benzamide.

20

9. A method for associating a particular disease or condition with a particular nuclear receptor, comprising the step of arylating a cysteine in said receptor.

25 10. The method of Claim 9 wherein said cysteine is within the ligand-binding domain of said receptor.

11. The method of Claim 9 wherein said arylating comprises binding a compound of Claim 1 to said nuclear receptor.

30

12. The method of claim 9 wherein said receptor is PPARgamma, LXR, or CAR.

13. A method for inhibiting the activity of a nuclear receptor comprising the step of arylating a cysteine residue within the ligand-binding domain of said receptor.

5

14. The method of Claim 13 wherein said step of arylation comprises binding a compound of Claim 1 to said ligand-binding domain.

10

15. The method of Claim 14 wherein said receptor is PPARgamma, LXR, or CAR.

15

16. A method for the prevention or treatment of a particular disease or condition which has been associated with a particular nuclear receptor by the method of Claim 9, comprising administration of a therapeutically effective amount of a compound that activates or deactivates said receptor.

20

17. Use of a compound which activates or deactivates a particular nuclear receptor for the manufacture of a medicament for the treatment of a particular disease or condition which has been associated with a particular nuclear receptor by the method of Claim 9.

18. The method of Claim 16 or use according to claim 17 wherein said receptor is PPARgamma, LXR, or CAR.

INTERNATIONAL SEARCH REPORT

Int lional Application No

PCT/US 00/06537

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07C233/66 C07C233/65 C07C235/38 C07K14/705 A61P5/00
C07D221/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07C C07K C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, PAJ, CHEM ABS Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 310 760 A (WASHBURN WILLIAM N ET AL) 10 May 1994 (1994-05-10) column 8	1-8
X	NAGARAJAN K ET AL: "CONDENSED HETEROTRICYCLES*: AMINO & AMINOALKYLDIBENZUB,FU1,4OXAZEPIN- 11(10H)-ONES" INDIAN JOURNAL OF CHEMISTRY, IN, JODHPUR, vol. 12, 1 March 1974 (1974-03-01), pages 236-246, XP000650379 page 5	1-6
P, X	WO 99 32477 A (SCHERING AG) 1 July 1999 (1999-07-01) abstract	1-6
	-/-	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

30 June 2000

Date of mailing of the international search report

19/07/2000

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Rufet, J

INTERNATIONAL SEARCH REPORT

Int. J. Application No

PCT/US 00/06537

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 99 29661 A (ASTRA PHARMA PROD ;CLADINGBOEL DAVID (GB); MORTIMORE MICHAEL (GB);) 17 June 1999 (1999-06-17) example 53	1-6
X	PATENT ABSTRACTS OF JAPAN vol. 1997, no. 07, 31 July 1997 (1997-07-31) & JP 09 059236 A (DAI ICHI SEIYAKU CO LTD), 4 March 1997 (1997-03-04) abstract	1-6
X	HANS-G. BOIT: "Beilsteins Handbuch der organischen Chemie, 4th Edition, 3th Suppl., vol. 12 " 1972, SPRINGER - VERLAG, BERLIN. HEIDELBERG. NEW YORK XP002141466 page 507, paragraph 3	1-6
X	FRIEDRICH RICHTER: "Beilsteins Handbuch der organischen Chemie, 4th Edition, second Suppl., vol. 12" 1950, SPRINGER-VERLAG, BERLIN. GÖTTINGEN. HEIDELBERG XP002141467 page 153, paragraphs 11,12	1-8
X	CHEMICAL ABSTRACTS, vol. 116, no. 19, 11 May 1992 (1992-05-11) Columbus, Ohio, US; abstract no. 194121x, NEMAZANYI, A. G. ET AL.: "Synthesis and structure of 3-amino-4-heteroaryl-1(2H)isoquinolones." page 708; column 1; XP002141468 abstract & KHIM. GETEROTSIKL. SOEDIN., vol. 8, 1991, pages 1104-1106,	1-6
X	CHEMICAL ABSTRACTS, vol. 85, no. 11, 13 September 1976 (1976-09-13) Columbus, Ohio, US; abstract no. 77216n, EGE, GUENTER ET AL.: "Ring-fission of cyclic azo compounds,..." page 475; column 2; XP002141469 abstract & JUSTUS LIEBIGS ANNALEN DER CHEMIE., vol. 5, 1976, pages 946-968, WEINHEIM DE	1-6

-/-

INTERNATIONAL SEARCH REPORT

Int'l. Jonal Application No

PCT/US 00/06537

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHEMICAL ABSTRACTS, vol. 71, no. 11, 15 September 1969 (1969-09-15) Columbus, Ohio, US; abstract no. 49619p, OSIEKA, HANS: page 355; column 2; XP002141470 abstract & ZA 6 802 762 A (BADISCHE ANILIN- UND SODA FABRIK AG) 1 August 1968 (1968-08-01)	1-8
X	BROGGINI G ET AL: "A new Entry to '1,2,4!Triazolo'1,5-a!'1,4!benzodiazepin-6 -ones via Intramolecular Nitrilimine Cycloaddition to the Cyano Group" TETRAHEDRON,NL,ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, vol. 54, no. 49, 3 December 1998 (1998-12-03), pages 14859-14868, XP004142608 ISSN: 0040-4020 page 14860	1-6
A	WO 94 12880 A (STANFORD RES INST INT ;JOLLA CANCER RES FOUND (US)) 9 June 1994 (1994-06-09) claims 1-70	1,9
A	WO 94 15901 A (LIGAND PHARM INC) 21 July 1994 (1994-07-21) abstract; claims 1-11	1,9
A,P	US 5 952 382 A (BERNARDON JEAN-MICHEL) 14 September 1999 (1999-09-14) abstract	1,9
A	WO 97 19062 A (ALLERGAN INC) 29 May 1997 (1997-05-29) page 1 -page 4; claims 1-17	1,9
A	US 5 756 448 A (BAES MYRIAM I ET AL) 26 May 1998 (1998-05-26) abstract	1,9
A	WO 98 32444 A (BASS NATHAN M ;ELIAS PETER M (US); HANLEY KAREN (US); UNIV CALIFOR) 30 July 1998 (1998-07-30) abstract; claim 1	1,9

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1, 9-15, 17 partially

Present claims 9-15, 17 relate to an extremely large number of possible methods or uses. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the methods or uses claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. It is stressed that the expressions "particular disease" as well as "particular nuclear receptor" include a large number of possibilities, which render a complete search impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to methods associated to obesity disorders according to page 11 of the current application with the receptors PPAR, LXR and CAR.

Present claims 1, 17 relate to an extremely large number of possible compounds and their use. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds according to formulae IA or IB, wherein R2 has the definition given by claim 3. The compounds described by the examples have been also searched. It is stressed that the definition of R2 given in claim 1 contains merely thousands of possibilities, which render a complete search impossible.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/06537

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5310760 A	10-05-1994	US 5449691 A	12-09-1995
WO 9932477 A	01-07-1999	AU 1875999 A	12-07-1999
WO 9929661 A	17-06-1999	AU 1791399 A	28-06-1999
JP 09059236 A	04-03-1997	NONE	
ZA 6802762 A		NONE	
WO 9412880 A	09-06-1994	US 5552271 A	03-09-1996
		US 5466861 A	14-11-1995
		AU 700706 B	14-01-1999
		AU 5869394 A	22-06-1994
		BR 9307528 A	31-08-1999
		EP 0671005 A	13-09-1995
		JP 8506323 T	09-07-1996
		US 5824484 A	20-10-1998
		US 5837725 A	17-11-1998
WO 9415901 A	21-07-1994	AU 6225894 A	15-08-1994
		AT 187434 T	15-12-1999
		AT 177733 T	15-04-1999
		AU 675430 B	06-02-1997
		AU 4118893 A	18-11-1993
		AU 691477 B	21-05-1998
		AU 5586894 A	15-08-1994
		AU 5664294 A	28-02-1995
		BR 9306284 A	13-01-1998
		BR 9307784 A	14-11-1995
		CA 2133587 A	28-10-1993
		CA 2153235 A	21-07-1994
		CA 2153236 A	09-02-1995
		DE 69324043 D	22-04-1999
		DE 69324043 T	01-07-1999
		DE 69327255 D	13-01-2000
		DE 69327255 T	30-03-2000
		EP 0637297 A	08-02-1995
		EP 0678086 A	25-10-1995
		EP 0678087 A	25-10-1995
		EP 0983991 A	08-03-2000
		EP 0983992 A	08-03-2000
		ES 2139063 T	01-02-2000
		ES 2129115 T	01-06-1999
		GR 3030104 T	30-07-1999
		JP 8511027 T	19-11-1996
		JP 8505852 T	25-06-1996
		JP 8505359 T	11-06-1996
		MX 9306627 A	29-07-1994
		MX 9306629 A	29-07-1994
		NO 943943 A	21-12-1994
		WO 9321146 A	28-10-1993
		WO 9504036 A	09-02-1995
		WO 9415902 A	21-07-1994
		US 5962731 A	05-10-1999
		US 5780676 A	14-07-1998
		US 6043279 A	28-03-2000

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/06537

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5952382 A	14-09-1999	FR 2733684 A	08-11-1996
		AU 691105 B	07-05-1998
		AU 5196496 A	19-12-1996
		BR 9601630 A	31-03-1998
		CA 2175643 A	04-11-1996
		EP 0740937 A	06-11-1996
		HU 9601161 A	28-05-1997
		JP 2968205 B	25-10-1999
		JP 9020655 A	21-01-1997
		NO 961742 A	04-11-1996
		PL 314046 A	12-11-1996
		ZA 9603413 A	06-11-1996
WO 9719062 A	29-05-1997	US 5663357 A	02-09-1997
		AU 715624 B	03-02-2000
		AU 1055197 A	11-06-1997
		BR 9611615 A	25-05-1999
		CA 2238310 A	29-05-1997
		CN 1207727 A	10-02-1999
		EP 0862555 A	09-09-1998
		HU 9901993 A	28-10-1999
		JP 2000500498 T	18-01-2000
		NZ 323574 A	28-10-1999
		PL 326925 A	09-11-1998
		US 6051713 A	18-04-2000
US 5756448 A	26-05-1998	US 5917048 A	29-06-1999
		AT 172247 T	15-10-1998
		AU 684458 B	18-12-1997
		AU 3728393 A	13-09-1993
		CA 2117491 A	02-09-1993
		DE 69321580 D	19-11-1998
		DE 69321580 T	17-06-1999
		EP 0628054 A	14-12-1994
		JP 7504416 T	18-05-1995
		WO 9317041 A	02-09-1993
		US 5710017 A	20-01-1998
		US 5686574 A	11-11-1997
WO 9832444 A	30-07-1998	AU 5928898 A	18-08-1998
		BR 9806989 A	14-03-2000
		CN 1248916 T	29-03-2000
		PL 334840 A	27-03-2000
		US 6060515 A	09-05-2000